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Origin of B chromosomes in *Characidium alipioi* (Characiformes, Crenuchidae) and its relationship with supernumerary chromosomes in other *Characidium* species

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Abstract

B chromosomes are apparently dispensable components found in the genomes of many species that are mainly composed of repetitive DNA sequences. Among the numerous questions concerning B chromosomes, the origin of these elements has been widely studied. To date, supernumerary chromosomes have been identified in approximately 60 species of fish, including species of the genus *Characidium* Reinhardt, 1867 in which these elements appear to have independently originated. In this study, we used molecular cytogenetic techniques to investigate the origin of B chromosomes in a population of *Characidium alipioi* Travassos, 1955 and determine their relationship with the extra chromosomes of other species of the genus. The results showed that the B chromosomes of *C. alipioi* had an intraspecific origin, apparently originated independently in relation to the B chromosomes of *C. gomesi* Travassos, 1956 *C. pterostictum* Gomes, 1947 and *C. oiticicai* Travassos, 1967, since they do not share specific DNA sequences, as well as their possible ancestral chromosomes and belong to different phylogenetic clades. The shared sequences between the supernumerary chromosomes and the autosommal sm pair indicate the origin of these chromosomes.

Keywords

Microdissection, Chromosome painting, FISH, B chromosomes

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Introduction

B or supernumerary chromosomes are extra genomic elements in addition to the standard chromosomal set (A) and are found in approximately 15% of eukaryotic organisms (Camacho 2005). In general, B chromosomes are derived from the A chromosomes of their own species (intraspecific origin) or closely related species (interspecific origin) (Banaei-Moghaddam et al. 2015). The intraspecific origin of B chromosomes was demonstrated in maize (Lamb et al. 2005; Peng et al. 2011), locusts (Teruel et al. 2010), rye (Martis et al. 2012) and fishes (Mestriner et al. 2000; Silva et al. 2013; Valente et al. 2014; Utsunomia et al. 2016). On the other hand, there are also cases in which B chromosomes may have arisen spontaneously in response to new genomic conditions such as interspecific hybridization, which has been observed in grasses of the genus *Coix* Linné, 1753 (Sapre and Deshepande 1987), the fish *Poecilia formosa* Girard, 1859 (Schartl et al. 1995) and the wasp *Nasonia vitripennis* Walker, 1836 (McAllister and Werren 1997).

A significant evolutionary feature of B chromosomes is the accumulation of repetitive DNA sequences (Camacho 2005; Houben et al. 2014; Banaei-Moghaddam et al. 2015). The cytogenetic and/or cytogenomic data about this type of DNA have been very informative for understanding the origin and evolution of B chromosomes. The findings using fluorescence *in situ* hybridization (FISH) techniques and genomic sequences analysis showed the intraspecific origin of B chromosomes and the presence and expression of intact genes in these elements in fishes (Silva et al. 2013, 2016; Utsunomia et al. 2016; Valente et al. 2014), as also observed in rye (Banei-Moghaddam et al. 2015).

Among genomes of fish species bearing B chromosomes, the genus *Characidium* Reinhardt, 1867 exhibits interesting cytogenetic features; the B chromosomes probably originated independently in the different species of this group, whereas the heteromorphic ZZ/ZW sex chromosomes seem to have originated once in the genus. Therefore, the B chromosomes of *C. oiticicai* Travassos, 1967 originated interspecifically, whereas in *C. gomesi* Travassos, 1956 and *C. pterostictum* Gomes, 1947, these elements have an intraspecific origin from the sex chromosomes (Pansonato-Alves et al. 2014, Serrano et al. 2016). In the present study, a novel occurrence of B chromosomes is described for *C. alipioi*, a species in which these elements were not found until now. In addition, the origin and evolution of these elements were studied using conventional cytogenetic techniques, including C-banding, microdissection, chromosome painting and fluorescence *in situ* hybridization with repetitive DNA probes.

Material and methods

Origin of the fishes/individuals, karyotype analysis and DNA extraction

A total of 19 *C. alipioi* samples were analyzed (9 females and 10 males) from the Ribeirao Grande river, Paraíba do Sul River Basin, Pindamonhangaba, São Paulo (22°49'00.3"S 45°25'23.7") (Table 1). The animals were collected in accordance with Brazilian envi-

Table 1. B chromosome polymorphisms in *Characidium alipioi*. Samples: number of males (M) and females (F) analyzed. Prevalence: the total and sex-specific percentage of individuals carrying B chromosomes.

Samples	Samples number with up to:					Prevalence
	50	**51**	**52**	**53**	**54**	
9 F	5	2	0	1	1	44%
10 M	5	3	1	0	1	50%
19 Total	**10**	**5**	**1**	**1**	**2**	**45%**

ronmental laws for the permission to collect issued by MMA/IBAMA/SISBIO, number 3245. The collection procedures, maintenance and analysis of the animals were performed in accordance with the international regulations for animal experiments, followed by the Universidade Estadual Paulista (CEEAA/IBB/UNESP protocol number 304). The samples were identified and deposited into the fish collection of the Biology and Genetics Laboratory of Fish at Botucatu, São Paulo, Brazil, under number 22287.

To perform the cytogenetic preparations, the animals were anesthetized and dissected, and mitotic chromosome preparations were obtained following the protocol of Foresti et al. (1981). C-banding was performed according to the protocol described by Sumner (1972). Chromosome morphology was determined according to Levan et al. (1964), and the chromosomes were classified as metacentric (m), submetacentrics (sm) and subtelocentric (st) and organized in the karyotype by descending size.

DNA extraction was performed using the Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions.

Mitochondrial DNA analysis

Amplification and partial sequencing of cytochrome oxidase c subunit 1 (COI) and cytochrome b (Cyt B) was performed to identify the specimens. The primers used were as follows: Cyt BL 14841 (5'-CCA TCC CAA ATC ACT GCA TGA TGA AA-3 ') and Cyt BH 15915b (5'-AAC CTC TCT CGA GCT GAT TACAAG AC -3') (Kocher et al. 1989) for Cyt B and COI L6252-Asn (5'-AAG GCG GGG AAA GCC GCC GCA G -3 ') and H7271-COXI (5'-TCC TAT GCC GAA GTA TGG TTC TTT T 3') for COI (Melo et al. 2011). The sequences were analyzed using Geneious Pro v8.05 software, and the alignment was performed with the algorithm MUSCLE (Edgar 2004). The average distances between the sequences were calculated using the "pairwise deletion" option in MEGA 4.0 software (Tamura et al. 2007).

Microdissection and preparation of chromosomal probe

Chromosome microdissection was performed in an Eppendorf TransferMan NK2 micromanipulator coupled with a Zeiss Axiovert 100 microscope. For chromosome

painting, ten B chromosomes were microdissected from the cytogenetic preparations of the samples from each species (*C. alipioi*, *C. gomesi* and *C. oiticicai)* carrying one extra chromosome. The probes for *C. alipioi*, *C. gomesi* and *C. oiticicai* denoted CaB, CgB and CoB, respectively.

Microdissected DNA from each species was placed into a tube containing 9 μL of DNase-free ultrapure water and amplified using the GenomePlex Single Cell Whole Genome Amplification Kit (wga4 Sigma) (Gribble et al. 2004). After the initial amplification, the DNA probes CaB, CgB and CoB were generated and were then labeled with Digoxigenin-11-dUTP (Roche Applied Science) using the GenomePlex Whole Genome Amplification Reamplification Kit (wga3 Sigma) according to the manufacturer's protocol.

Repetitive DNA probe

Probes for 18S and 5S rDNA, U2 snDNA and histone H3 genes were obtained using PCR from the *C. alipioi* genome with previously described primers. Sequences for 5S and 18S rDNA were amplified using the *primers* 5SA (5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3') and 5SB (5'-TAG ACT TCT GGG TGG CCA AAG GAA TCA-3') (Pendás et al. 1995), 18S6F (5'-CTC TTT CGA GGC CCT GTA AT-3') and 18S6R (5'-CAG CTT TGC AAC CAT ACT CC-3') (Utsunomia et al. 2016). To amplify the H3 histone gene were utilized the following *primers*, H3F (5'-ATG GCT CGT ACC AAG CAG ACV GC-3') and H3R (5'-ATA TCC TTR GGC ATR ATR GTG AC-3') (Colgan et al. 1998). The U2 snDNA was amplified by the *primers* U2F (5'-ATC GCT TCT CGG CCT TAT G-3') and U2R (5'-TCC CGG CGG TAC AAT TGC A-3') (Silva et al. 2015).. The 18S rDNA, U2 snDNA and histone H3 probes were labeled with Digoxigenin-11-dUTP (Roche), and the 5S rDNA probe was labeled with biotin-16-dUTP (Roche). Oligonucleotide probes sequences containing microsatellite $(CA)_{15}$, $(GA)_{15}$ and $(GAG)_{10}$ were labeled directly with TAMRA during the synthesis process by Sigma according to methods described by Kubat et al. (2008).

Fluorescent in situ hybridization (FISH)

For the FISH experiments, the prehybridization conditions were performed according to procedures described by Pinkel et al. (1986). Posthybridization washes were performed according to the applied probe: (i) the slides probed with rDNAs, snDNA, histone and B chromosome probes were washed in 0.2× SSC/15% formamide for 20 min at 42°C, followed by a second wash in 0.1× SSC for 15 min at 60°C and a final wash at room temperature in 4× SSC/0.5% Tween for 10 min; probe detection was performed using avidin-FITC and anti-digoxigenin-rhodamine; and (ii) the slides probed with oligonucleotides were washed in 2× SSC for 5 min, followed by a second wash in 1×

PBS for 1 min. Chromosomes were counterstained with DAPI (Vector Laboratories, Burlingame, Calif., USA). The images were digitally captured using Image Pro Plus 6.0 software (Media Cybernetics) with the appropriate filters on an epifluorescence microscope (Olympus BX61) equipped with an Olympus DP70 camera. The final composition of the images was performed using Adobe Photoshop CS6 image editor software with the image and uniform size scales.

Results

Analysis of mitochondrial DNA

Analysis of mitochondrial DNA was performed in order to make a correct identification of the specimens, besides that, the position of the clades could be informative to discuss aspects of the origin of the B chromosomes in the genus. The average distance analysis of the COI and CytB sequences obtained in this study and other species taken from GenBank showed high similarity between the *C. alipioi* sequences of Santa Bárbara do Tugúrio-MG and the specimens analyzed here (Suppl. materials 1, 2), which confirmed the taxonomic status of the analyzed samples (Figure 1).

Chromosomal analysis

The analyzed *C. alipioi* individuals showed diploid chromosome number 2n = 50 and karyotype composed of 32 m + 18 sm with heteromorphic ZZ/ZW sex chromosomes, which was similar to the findings in other species of the genus (Figure 2). In addition, cells bearing 0-4 B chromosomes were observed in 45% of the individuals (Table 1). The B chromosomes were mitotically unstable, once the number of these chromosomes can variete between cells of the same individual. C-banding showed constitutive heterochromatin blocks in the pericentromeric region of all chromosomes and a conspicuous distal block in the longer arm of the Z chromosome. The W and B chromosomes were entirely heterochromatic (Figure 2b).

Chromosome painting

Chromosome painting performed in the *C. alipioi* chromosomes with the CaB probe showed signals on the entire length of the B chromosomes and the pericentromeric region of the sm chromosome pair nº 19 of the A complement (Figure 3a). On the other hand, this same probe did not reveal any signals on the *C. gomesi* and *C. oiticicai* chromosomes (Figure 3b and c). Similar findings were observed by hybridization with the CoB probe in *C. alipioi* chromosomes (Figure 3e). Conversely, the CgB probe showed hybridization signals on the W chromosome of *C. alipioi* (Figure 3d).

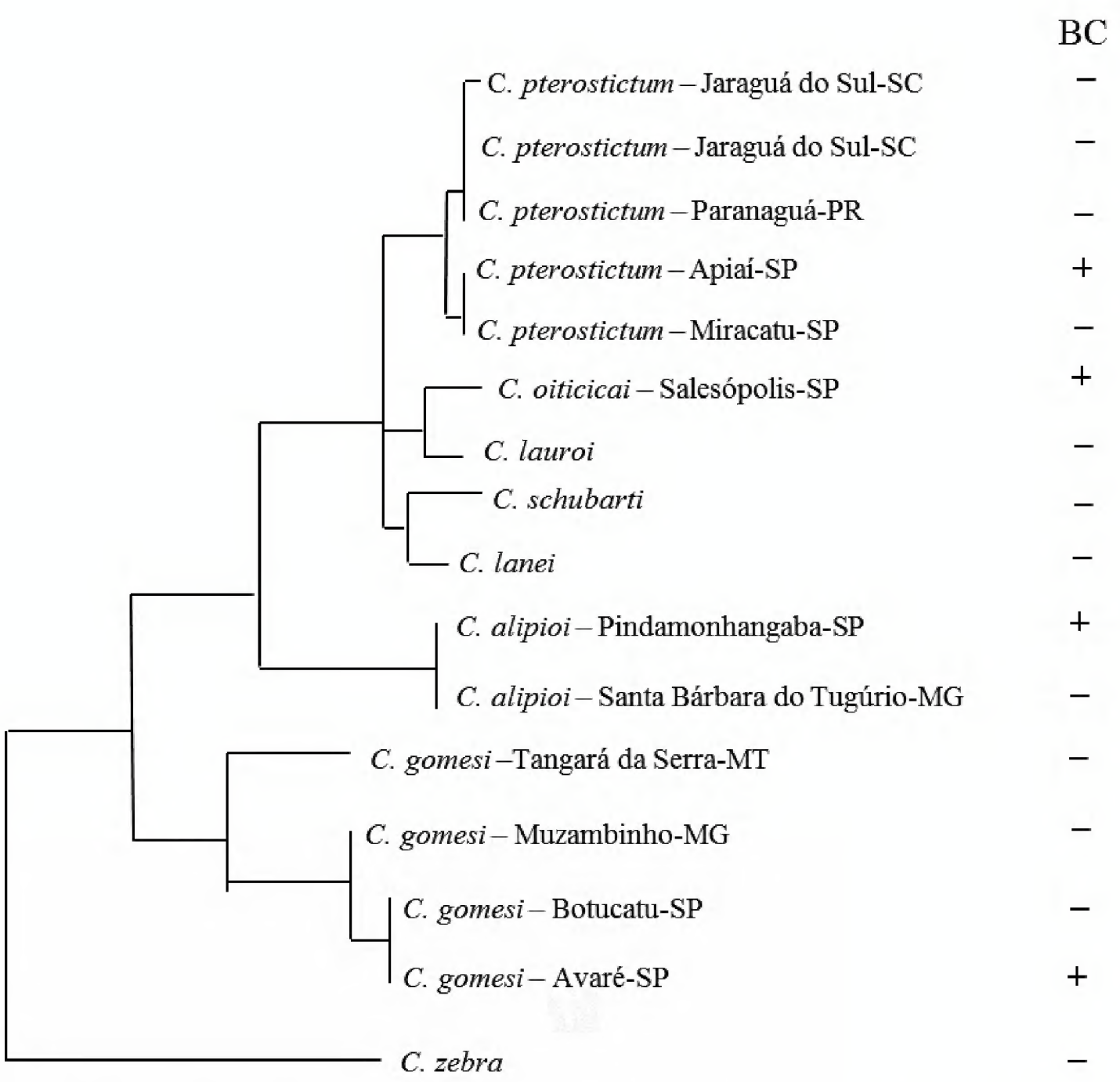
BC
C. pterostictum – Jaraguá do Sul-SC
C. pterostictum – Jaraguá do Sul-SC
C. pterostictum – Paranaguá-PR
C. pterostictum – Apiaí-SP
C. pterostictum – Miracatu-SP
C. oiticicai – Salesópolis-SP
C. lauroi
C. schubarti
C. lanei
C. alipioi – Pindamonhangaba-SP
C. alipioi – Santa Bárbara do Tugúrio-MG
C. gomesi – Tangará da Serra-MT
C. gomesi – Muzambinho-MG
C. gomesi – Botucatu-SP
C. gomesi – Avaré-SP
C. zebra
– – – – + – + – – – + – – – + – –

Figure 1. *Characidium* phylogeny adapted from Pansonato-Alves et al. (2014) and positioned for the population analyzed here. BC: presence or absence of B chromosomes.

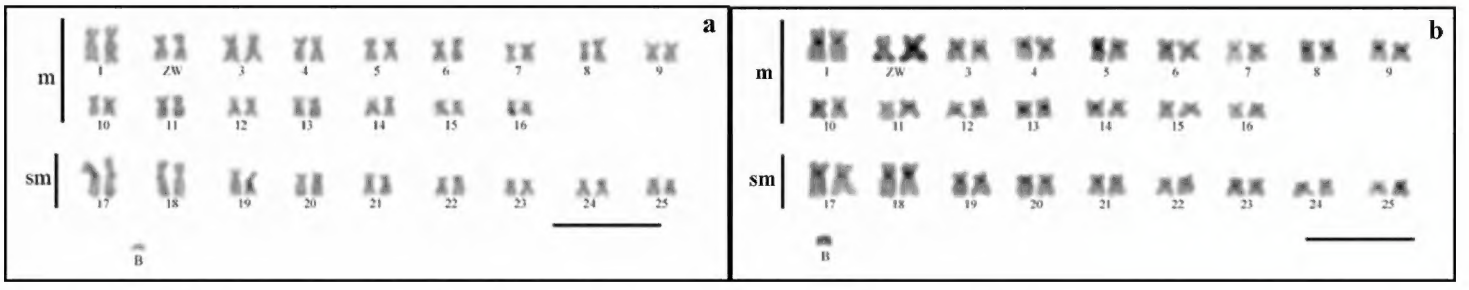

Figure 2. *Characidium alipioi* karyotypes arranged from mitotic metaphases after to conventional Giemsa-staining (**a**) and C-banding (**b**). Bar = 10 μm.

Distribution of repetitive DNA probes

FISH experiments on the *C. alipioi* chromosome preparations using a 18S rDNA probe revealed sites of this gene in the terminal position of autosomal pair No. 18,

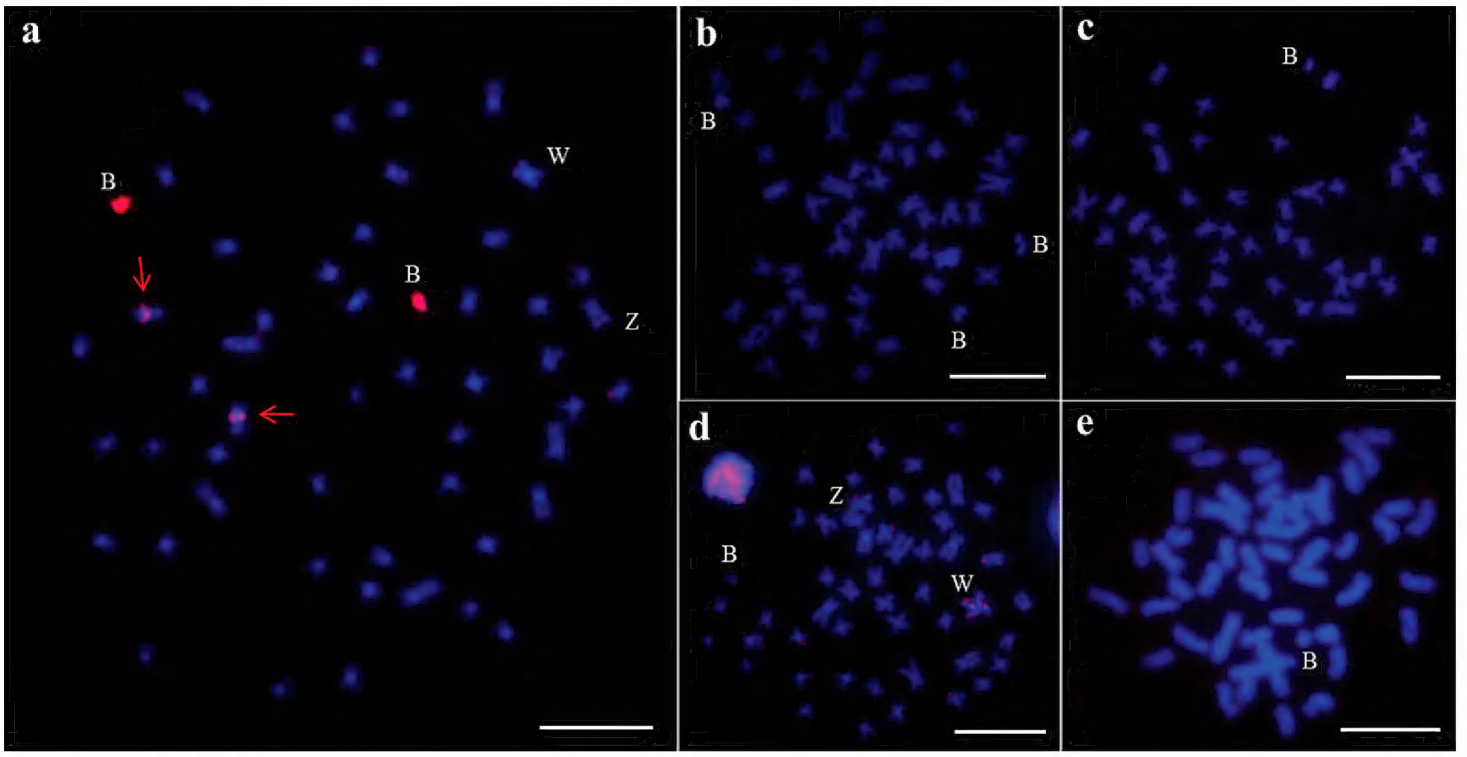

Figure 3. Cross-species chromosome painting. CaB probe in *Characidium alipioi* (**a**), *C. gomesi* (**b**) and *C. oiticicai* (**c**) and with the CgB (**d**) and CoB (**e**) probes in *C. alipioi*.

whereas the 5S rDNA was mapped in the pericentromeric region of chromosome pair No. 20 (Figure 4a). Histone H3 sites were found in the m chromosome pair No. 10 (Figure 4b), whereas the U2 snRNA gene was located on the sm chromosomes pair No. 17 (Figure 4c).

FISH with probes containing the microsatellite sequences $(CA)_{15}$ and $(GA)_{15}$ in the *C. alipioi* chromosomal preparations showed similar patterns of distribution with preferential accumulation in the terminal regions of the chromosomes, except for the W and B chromosomes (Figure 5a and b), which had lower abundance and weak hybridization signals. Furthermore, the intense signals of the microsatellite $(GAG)_{10}$ showed preferential accumulation on the W and B chromosomes of this species (Figure 5c).

Discussion

The occurrence of B chromosomes in *Characidium alipioi* genome was revealed for the first time in this study, particularly since the population of *C. alipioi* of Santa Bárbara do Tugúrio-MG analyzed by Pansonato-Alves et al. (2014) showed no extra chromosomes. The presence of these elements in only certain populations was observed in other species of the genus *Characidium* such as *C. zebra*, *C. pterostictum* and *C. gomesi* (Pansonato-Alves et al. 2014). Our data does not allow to conclude about the dynamics or relationship of these chromosomes between populations; however, at least two mechanisms, which are based on the findings of two model organisms have been proposed to be involved in the absence of B chromosomes in certain populations: geographical barriers that limit the spread of individuals with these elements in

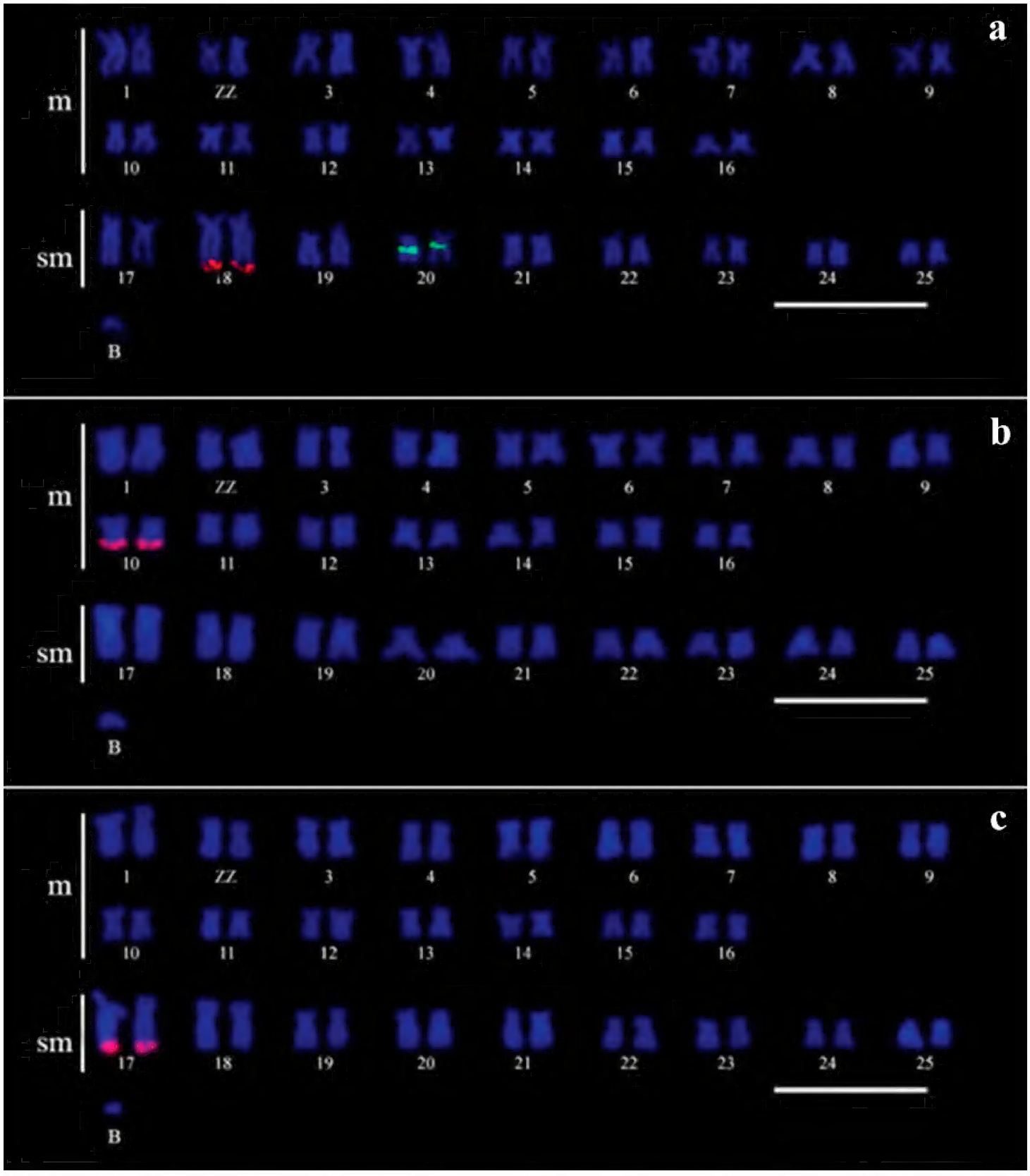

Figure 4. Karyotypes of *Characidium alipioi* arranged from mitotic metaphases after FISH with repetitive DNA probes. **a** 18S and 5S rDNA probes **b** histone H3 probe, and **c** U2 snRNA probe. Bar =10 μm.

Eyprepocnemis plorans Charpentier, 1825 (Cabrero et al. 1997, Manrique-Poyato et al. 2015) and the close relationship between temperature and rainfall factors that could influence the variations in the presence of B chromosomes, as observed in *Myrmeleotettix maculatus* Thunberg, 1815 (Hewitt and John 1967; Hewitt and Ruscoe 1971).

The individuals of the same species and location included in this study were analyzed in a previous study by Centofante et al. (2003); however, these authors did not detect B chromosomes in their analysis. Although cytogenetic studies in *Characidium* have revealed the occurrence of supernumerary chromosomes restricted to certain

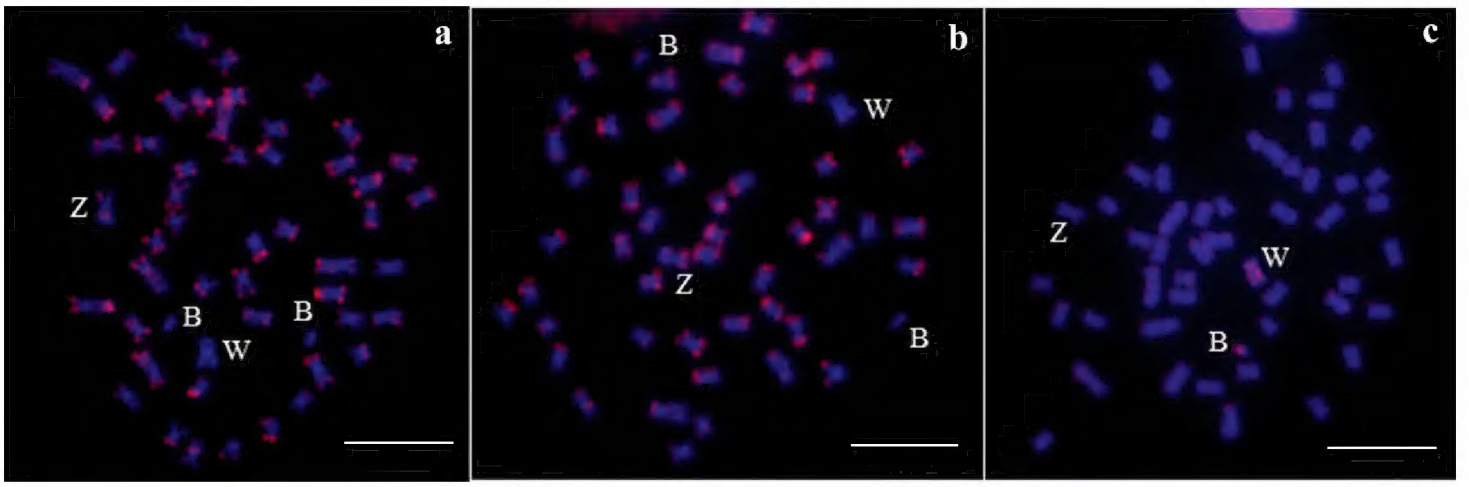

Figure 5. Mitotic metaphases after FISH with the microsatellites. **a** $(GA)_{15}$ **b**) $(CA)_{15}$ and **c**) $(GAG)_{10}$. Note the accumulation of (GAG) 10 in the B and W chromosomes.

populations of some species (Pansonato-Alves et al. 2014), it should be noted that the geographical proximity of the sampling sites and the time between the present analysis and the analysis by Centofante et al. (2003) suggest that the two samples do not belong to different populations. Similarly, it can't be stated that the B chromosomes have arisen in this population after the first study. However, because some individuals with no B chromosomes were also identified in the present study, it is likely that the samples analyzed in the 2003 study were formed by individuals who do not carry these extra elements.

Chromosome painting using the B chromosomes of *C. alipioi* (CAB) as the probe indicated shared sequences between the A and B complements and, more specifically, the pericentromeric region of a sm chromosome pair (pair No. 19). This result probably reflects a relationship between these chromosomes and intraspecific origin of the supernumerary chromosomes in genome of this species and that pair No. 19 is likely the chromosome of its origin. Previous studies have identified the origin of B chromosomes from the A complement of the host species (Bugrov et al. 2003; De Jesus et al. 2003; Lamb et al. 2005; Teruel et al. 2010; Peng et al. 2011; Silva et al. 2014; Valente et al. 2014; Utsunomia et al. 2016); however, in a few cases it was possible to identify the ancestral chromosome, such as in the grasshopper (Teruel et al. 2010; Bueno et al. 2013) and in two fish species *Astyanax paranae* Eigenmann, 1914 (Silva et al. 2014) and *Moenkhausia sanctaefilomenae* Steindachner, 1907 (Utsunomia et al. 2016).

The intraspecific origin of B chromosomes has been reported in other species of the genus *Characidium*, namely *C. pterostictum*, *C. gomesi* and *Characidium* sp. aff. *C. vidalli* (Pansonato-Alves et al. 2014; Schacchetti et al. 2015). However, because in these species this chromosome originated from the Z and W chromosomes and the CaB probe in the present study showed no signals on these chromosomes, these supernumerary elements do not seem to share the same ancestral supernumerary chromosomes that are present in *C. alipioi*. In addition, the *C. alipioi* B chromosomes showed no homology with the supernumeraries of *C. gomesi* and *C. oiticicai*, which are two species whose B chromosomes are apparently composed of different types of repetitive DNA and have different origins

(Pansonato-Alves et al. 2014). Therefore, it possible that the supernumerary elements in *C. alipioi* originated independently from the other types of B chromosomes reported in the representatives of this genus, and the phylogenetic position of these species at different clades (Figure 1) supports this assumption. However, these conclusions should be made with caution because the probes obtained by microdissection are composed of anonymous sequences, and the amplification method (GenomePlex) may favor specific sequences of repetitive DNA that are present in the B chromosomes and absent on other A chromosomes. In this context, it cannot be ruled out that other sequences shared between A and B chromosomes are not represented in the probes used in this study, which was previously noted by Pansonato-Alves et al. (2014).

Hybridizations with microsatellite DNA sequences demonstrated the presence of these repetitive elements in B chromosomes. $(GA)_{15}$ and $(CA)_{15}$ are both dispersed with conspicuous blocks in the terminal regions of the A chromosomes and are less abundant in the supernumerary chromosomes. Moreover, a clear accumulation was observed with respect to the $(GAG)_{10}$ sequence. Notably, the hybridization with the $(GAG)_{10}$ probe revealed a preferential accumulation in B and W chromosomes in *C. alipioi*. Similarly, microsatellites were detected in the B chromosomes of maize (Ananiev et al. 2005), rye (Langdon et al. 2000) and locusts (Milani and Cabral-de-Mello 2014; Ruiz-Ruano et al. 2015). Given that the CaB probe did not paint the *C. alipioi* sex chromosomes, a possible explanation for the accumulation of $(GAG)_{10}$ in the B and W chromosomes would be that the CaB probe does not contain this microsatellite, which probably occurred due to the amplification method used in this study, as mentioned above. However, if it is considered that the accumulation mechanisms of this type of repetitive DNA permit its fixation on certain chromosomes due to its non-recombinant nature and preferential accumulation in heterochromatic regions, as observed in other studies (Cuadrado and Jouve 2011, Lohe et al. 1993, Scacchetti et al. 2015), then the distribution of microsatellites in these chromosomes may not reflect the aspects of its origin, but its accumulation after the appearance of the B chromosomes.

Our present results extend the knowledge of the structure and composition of B chromosomes between representatives of the *Characidium* genus, particularly in *C. alipioi*. In addition, the shared sequences between the A and B chromosomes of this species suggests an intraspecific origin of these chromosomes that is independent from the B chromosomes of other congeneric species. These observations reinforce the idea that this fish group is an interesting model to study the origin and structure of B chromosomes.

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References

Ananiev E, Chamberlin MA, Klaiber J, Svitashev S (2005) Microsatellite megatracts in the maize (*Zea mays* L.) genome. Genome 48: 1061–1069. https://doi.org/10.1139/g05-061

Banaei-Moghaddam AM, Martis MM, Macas J, Gundlach H, Himmelbach A, Altschmied L, Mayer KFX, Houben A (2015) Genes on B chromosomes: Old questions revisited with new tools. Biochimica et Biophysica Acta (BBA)- Gene Regulatory Mechanisms 1849(1): 64–70. https://doi.org/10.1016/j.bbagrm.2014.11.007

Bueno D, Palacios-Gimenez OM, Cabral-de-Mello DC (2013) Chromosomal mapping of repetitive DNAs in *Abracris flavolineata* reveal possible ancestry for the B chromosome and surprisingly H3 histone spreading. PLoS ONE 8: e66532. https://doi.org/10.1371/journal.pone.0066532

Bugrov AG, Karamysheva TV, Pyatkova MS et al. (2003) B chromosomes of the *Podisma sapporensis* Shir (Orthoptera, Acrididae) analyzed by chromosome microdissection and FISH. Folia Biologica (Krakow) 51: 1–11.

Cabrero J, Lo M, Go R, Castro A, Marti A, Camacho J (1997) Geographical distribution of B chromosomes in the grasshopper *Eyprepocnemis plorans*, along a river basin, is mainly shaped by non-selective historical events. Chromosome Research 5(3): 194–198. https://doi.org/10.1023/A:1018499015091

Camacho JPM (2005) B chromosomes. In: Gregory TR (Ed.) The evolution of the genome. Elsevier, San Diego, 223–286. https://doi.org/10.1016/b978-012301463-4/50006-1

Centofante L, Bertollo LAC, Buckup PA, Moreira-Filho O (2003) Chromosomal divergence and maintenance of sympatric *Characidium* fish species (Crenuchidae, Characidiinae). Hereditas 138: 213–218. https://doi.org/10.1034/j.1601-5223.2003.01714.x

Colgan D, McLauchlan A, Wilson G, Livingston S (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Australian Journal of Zoology 46: 419–43. https://doi.org/10.1071/ZO98048

Cuadrado A, Jouve N (2011) Novel simple sequence repeats (SSRs) detected by ND-FISH in heterochromatin of *Drosophila melanogaster*. BMC Genomics 12: 205. https://doi.org/10.1186/1471-2164-12-205

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797. https://doi.org/10.1093/nar/gkh340

Foresti F, Almeida-Toledo LF, Toledo-Filho SA (1981) Polymorphic nature of nucleolus organizer regions in fishes. Cytogenetics and Cell Genetics 31(3): 37–144. https://doi.org/10.1159/000131639

Gribble S, Ng BL, Prigmore E, Burford DC, Carter NP (2004) Chromosome paints from single copies of chromosomes. Chromosome Research 12: 143–151. https://doi.org/10.1023/B:CHRO.0000013167.12527.f0

Hewitt G, John B (1967) The B-chromosome system of *Myrmeleotettix maculatus*. Chromosoma 21(2): 140–62. https://doi.org/10.1007/BF00343641

Hewitt G, Ruscoe C (1971) Changes in microclimate correlated with a cline for B-chromosomes in the grasshopper *Myrmeleotettix maculatus* (Orthoptera: Acrididae). The Journal of Animal Ecology 40(3): 753–65. https://doi.org/10.2307/3448

Houben A, Banaei-Moghaddam AM, Klemme S, Timmis JN (2014) Evolution and biology of supernumerary B chromosomes. Cellular and Molecular Life Sciences 71: 467–478. https://doi.org/10.1007/s00018-013-1437-7

Jesus CM, Galetti Jr PM, Valentini SR, Moreira-Filho O (2003) Molecular characterization and chromosomal localization of two families of satellite DNA in *Prochilodus lineatus* (Pisces, Prochilodontidae), a species with B chromosomes. Genetica 118(1): 25–32. https://doi.org/10.1023/A:1022986816648

Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S et al. (1989) Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences 86: 6196–6200. https://doi.org/10.1073/pnas.86.16.6196

Kubat Z, Hobza R, Vyskot B, Kejnovsky E (2008) Microsatellite accumulation on the Y chromosome of *Silene latifolia*. Genome 51: 350–356. https://doi.org/10.1139/G08-024

Lamb JC, Kato A, Birchler JÁ (2005) Sequences associated with A chromosome centromeres are present throughout the maize B chromosome. Chromosoma 113(7): 337–349. https://doi.org/10.1007/s00412-004-0319-z

Langdon T, Seago C, Jones RN, Ougham H, Thomas H, Forster JW, Jenkins G (2000) De novo evolution of satellite DNA on the rye B chromosome. Genetics 154: 869–884. www.genetics.org/content/genetics/154/2/869.full.pdf

Levan A, Fredga K, Sandbreg AA (1964) Nomeclature for centromeric position on chromosomes. Hereditas 52: 201–220. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x

Lohe AR, Hilliker AJ, Roberts PA (1993) Mapping simple repeated DNA sequences in heterochromatin of *Drosophila melanogaster*. Genetics 134: 1149–1174. http://www.genetics.org/content/genetics/134/4/1149.full.pdf

Manrique-Poyato MI, López-León MD, Cabrero J, Gómez R, Perfectti F, Camacho JPM (2015) Geographical barriers impeded the spread of a parasitic chromosome. PLoS ONE 10(6): e0131277. https://doi.org/10.1371/journal.pone.0131277

Martis MM, Klemme S, Banaei-Moghaddam AM, Blattner FR, Macas J, Schmutzer T, Scholz U et al. (2012) Selfish supernumerary chromosome reveals its origin as a mosaic of host genome and organelar sequences. Proceedings of the National Academy of Sciences 109(33): 13343–13346. https://doi.org/10.1073/pnas.1204237109

McAllister BF, Werren JH (1997) Hybrid origin of a B chromosome (PSR) in the parasitic wasp *Nasonia vitripennis*. Chromosoma 106(4): 243–53. https://doi.org/10.1007/s0044120050245

Melo BF, Benine RC, Mariguela TC, Oliveira C (2011) A new species of *Tetragonopterus* Cuvier, 1816 (Characiformes: Characidae: Tetragonopterinae) from the rio Jari, Amapá, northern Brazil. Neotropical Ichthyology 9(1): 49–56. https://doi.org/10.1590/S1679-62252011000100002

Mestriner CA, Galetti Jr. PM, Valentini SR, Ruiz IRG, Abel LDS, Moreira-Filho O, Camacho JPM (2000) Structural and functional evidence that a B chromosome in the characid fish *Astyanax scabripinnis* is an isochromosome. Heredity 85: 1–9. https://doi.org/10.1046/j.1365-2540.2000.00702.x

Milani D, Cabral-de-Mello DC (2014) Microsatellite organization in the grasshopper *Abracris flavolineata* (Orthoptera: Acrididae) revealed by FISH mapping: remarkable spreading in

the A and B chromosomes. PLoS ONE 9(5): e97956. https://doi.org/10.1371/journal.pone.0097956

Pansonato-Alves JC, Serrano EA, Camacho JPM, Utsunomia R, Silva GJC, Oliveira C, Foresti F (2014) Single origin of sex chromosomes and multiple origins of B chromosomes in fish of the genus *Characidium*. PLoS ONE 9(9): e107169. https://doi.org/10.1371/journal.pone.0107169

Pendás AM, Morán P, Martínez JL, Garcia-Vásquez E (1995) Applications of 5S rDNA in Atlantic salmon, brown trout, and in Atlantic salmon x brown trout hybrid identification. Molecular Ecology 4: 275–276. https://doi.org/10.1111/j.1365-294X.1995.tb00220.x

Peng SF, Cheng YM (2011) Characterization of satellite CentC repeats from heterochromatic regions on the long arm of maize B-chromosome. Chromosome Research 19: 183–191. https://doi.org/10.1007/s10577-010-9183-2

Pinkel D, Straume T, Gray JW (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proceedings of the National Academy of Sciences 83: 2934–2938. https://doi.org/10.1073/pnas.83.9.2934

Ruiz-Ruano FJ, Cuadrado Á, Montiel EE, Camacho JPM, López-León MD (2015) Next generation sequencing and FISH reveal uneven and nonrandom microsatellite distribution in two grasshopper genomes. Chromosoma 124(2): 221–234. https://doi.org/10.1007/s00412-014-0492-7

Sapre A, Deshpande D (1987) Origin of B chromosomes in Coix L. through spontaneous interspecific hybridization. Journal of Heredity 78(3): 191–196.

Scacchetti PC, Utsunomia R, Pansonato-Alves JC, da Costa Silva GJ, Vicari MR, Artoni RF, Oliveira C, Foresti F (2015) Repetitive DNA Sequences and Evolution of ZZ/ZW Sex Chromosomes in *Characidium* (Teleostei: Characiformes). PLoS ONE 10(9): e0137231. https://doi.org/10.1371/journal.pone.0137231

Schartl M, Nanda I, Schlupp I, Wilde B, Epplen JT et al. (1995) Incorporation of subgenomic amounts of DNA as compensation for mutational load in a gynogenetic fish. Nature 373: 68–71. https://doi.org/10.1038/373068a0

Serrano EA, Araya-Jaime C, Suárez-Villota EY, Oliveira C, Foresti F (2016) Meiotic behavior and H3K4m distribution in B chromosomes of *Characidium gomesi* (Characiformes, Crenuchidae). Comparative Cytogenetics 10(2): 255–268. https://doi.org/10.3897/CompCytogen.v10i2.7939

Silva DMZA, Pansonato-Alves JC, Utsunomia R, Daniel SN, Hashimoto DT, Oliveira C, Porto-Foresti F, Foresti F (2013) Chromosomal organization of repetitive DNA sequences in *Astyanax bockmanni* (Teleostei, Characiformes): dispersive location, association and co-localization in the genome. Genetica 141(7-9): 329–336. https://doi.org/10.1007/s10709-013-9732-7

Silva DM, Utsunomia R, Pansonato-Alves JC, Oliveira C, Foresti F (2015) Chromosomal mapping of repetitive DNA sequences in five species of *Astyanax* (Characiformes, Characidae) reveals independent location of U1 and U2 snRNA sites and association of U1 snRNA and 5S rDNA. Cytogenetic and genome research 146(2): 144–152. https://doi.org/10.1159/000438813

Silva DMZA, Daniel SN, Camacho JPM, Utsunomia R, Ruiz-Ruano FJ, Penitente M, Pansonato-Alves JC, Hashimoto DT, Oliveira C, Porto-Foresti F, Foresti F (2016) Origin

of B chromosomes in the genus *Astyanax* (Characiformes, Characidae) and the limits of chromosome painting. Molecular Genetics and Genomics 1–12. https://doi.org/10.1007/s00438-016-1195-y

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research 75: 304–306. https://doi.org/10.1016/0014-4827(72)90558-7

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) Software Version 4.0. Molecular Biology and Evolution 24: 1596–1599. https://doi.org/10.1093/molbev/msm092

Teruel M, Cabrero J, Perfectti F, Camacho JPM (2010) B chromosome ancestry revealed by histone genes in the migratory locust. Chromosoma 119: 217–225. https://doi.org/10.1007/s00412-009-0251-3

Utsunomia R, Silva DMZA, Ruiz-Ruano FJ, Araya-Jaime C, Pansonato-Alves JC, Scacchetti PC, Hashimoto DT, Oliveira C, Trifonov VA, Porto-Foresti F (2016) Camacho JPM, Foresti. Uncovering the ancestry of B chromosomes in *Moenkhausia sanctaefilomenae* (Teleostei, Characidae). PLoS ONE 11(3): e0150573. https://doi.org/10.1371/journal.pone.0150573

Valente GT, Conte MA, Fantinatti BE, Cabral-de-Mello DC, Carvalho RF, Vicari MR, Kocher TD, Martins C (2014) Origin and evolution of B chromosomes in the cichlid fish *Astatotilapia latifasciata* based on integrated genomic analyses. Molecular Biology and Evololution 31: 2061–2072. https://doi.org/10.1093/molbev/msu148

Supplementary material 1

Table S1

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Data type: molecular data

Explanation note: Average distance between the CytB sequences of the *Characidium* species.

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Supplementary material 2

Table S2

Authors: Érica Alves Serrano, Ricardo Utsunomia, Patrícia Sobrinho Scudeller, Claudio Oliveira, Fausto Foresti

Data type: molecular data

Explanation note: Average distance between the COI sequences of the *Characidium* species.

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